REMARKS

Claims 23-27 have been rejected under 35 U.S.C. §§ 112, first paragraph, 102 or 103. Claim 28 has been withdrawn by the Examiner as directed to non-elected subject matter. Applicants expressly reserve the right to file a divisional application directed to the non-elected subject matter. Applicants have amended claims 23 and 24 to recite "an antibody specific to a Flagellin gene of *Salmonella* typhi". Support for this amendment can be found in the specification at, for example, page 8, line 8 to page 9 line 17. Thus, no new matter has been added by this amendment.

In view of the remarks below, Applicants respectfully request reconsideration and withdrawal of the asserted objections and rejections.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 23-27 have been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Specifically, the Examiner contends that "neither the specification nor originally presented claims provides support for a process for preparing an agglutination reagent for detecting typhoid comprising preparing a polyclonal-monospecific antibody."[1]

Applicants have deleted the recitation of "polyclonal-monospecific" from claim 23. Accordingly, withdrawal of this rejection is respectfully requested.

Rejection under 35 U.S.C. § 102

Claims 24-26 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Lim[2]. Applicants respectfully disagree for the reasons discussed below.

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[1] Office Action at page 7

[2] Lim *et al.*, "Detection of Group D Samonallae in Blood Culter Broth and of Soluble Antigen by Tube Agglutination Using an O-9 Monoclonal Antibody Latex Conjugate," J. OF CLIN. MICROBIOL., (July 1987) 25(7): 1165-1168 ("Lim").

Point I. Lim uses an O-9 system for antigen detection, not a Flagellin Gene

Claims 24 has been amended to recite "An agglutination reagent ... comprising a carboxylated latex particle coated with an antibody specific to a Flagellin gene of *Salmonella* typhi ...." In contrast, Lim used the O-9 monoclonal antibody from Wellcome Diagnostics.[3] "A limitation of the O-9 system is that infection caused by *S. Typhi*, the most important cause of enteric fever, cannot be distinguished from those of the other group D salmonellae; however, they may not be clinically important. Differentiation requires additional tests such as examining for the presence of d-H and Vi antigens in *S. Typhi* organisms."[4]

The O-9 antigen is present in the cell wall of the entire group D Salmonellae. In contrast, the claimed antibody specific to the Flagellin Gene is highly specific to *S-Typhi* (see Figure 1). Accordingly, Lim's method detects group D Salmonella. This would include *S. Typhi* and *S. paratyphi*. In contrast, the recited invention is specific to *S. typhi*. Thus, in order to diagnose *S. Typhi* using Lim's disclosure, an additional step would be required.

[3] Lim at page 1166, col. 1.
[4] Lim at page 1167, col. 2.

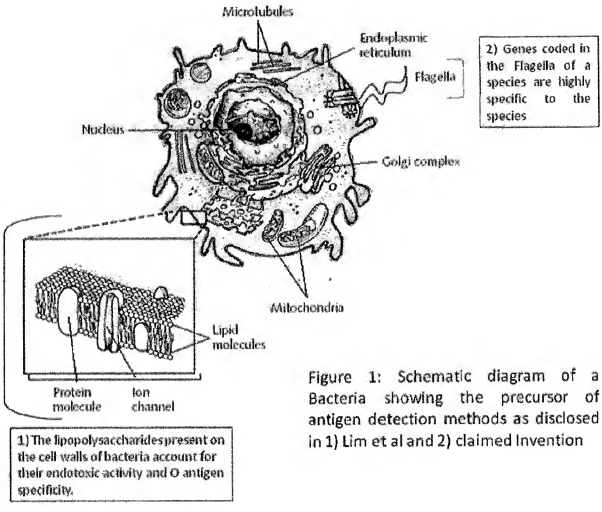


Figure 1: Schematic diagram of a Bacteria showing the precursor of antigen detection methods as disclosed in 1) Lim et al and 2) claimed Invention

Since Lim does not teach or suggest using the Flagellin gene and is not specific to *S. typhi*, it does not anticipate claim 24, or claims 25 and 26, which depend from claim 24.

Point II. Lim uses non-carboxylated latex particles, not carboxylated latex particles.

Claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. The reagent comprises 1% carboxylated latex particles coated with antibody specific to *Salmonella typhi*, suspended in storage buffer.

Lim used "[a] 1% suspension of latex particles (diameter, 0.797 μm; Sigma Chemical Co., Ltd. Poole, United Kingdom) was sensitized in 0.1 M glycine-0.9% sodium chloride buffer (pH 8.2) with an equal volume of *Salmonella* O-9 monoclonal immunoglobulin M (IgM) antibodies (7) ..."[5] Although this passage does not specifically state that the latex particles are carboxylated, the Examiner contends that they are without providing any evidence to support this contention. Instead, the Examiner improperly shifts the burden to the Applicants to prove a negative – why Lim's latex particles are not carboxylated. For this reason, a *prima facie* case of anticipation has not been established.

Notwithstanding this, Applicants have previously submitted print-outs from Sigma-Aldrich's website, which is believed to be the Sigma Chemical Co. referenced in Lim because Sigma-Aldrich is the resulting company from the merger of Sigma Chemical Co. and Aldrich Chemical Co. in 1975. The print-out lists all of the latex beads available by Sigma-Aldrich. They include amine modified polystyrene, carboxylate-modified polystyrene, deep blue dyed, sulfate modified polysterene and plain polystyrene beads. First, since Lim does not indicate that the latex beads are modified, the only reasonable assumption is that the beads are plain polystyrene. If they were something else, one would have expected Lim to expressly state how the beads were modified. Second, the only bead available from Sigma-Aldrich that matches the size of the particles described by Lim is a plain (or noncarboxylated) polystyrene bead. Sigma-Aldrich only offers one latex bead having a mean particular size of 0.8 μm, LB8. This product is not carboxylated. For these reasons, Applicants believe that Lim used LB8.

Since Lim does not teach using a carboxylated bead, it does not teach each and every limitation recited in claim 24. Claims 25 and 26, depend from and further limit claim 24 and also require utilization of a carboxylated bead. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

[5] Lim at p. 1165, col. 2.

Point III. Lim does not use thimerosal in its storage buffer

Claim 26 recites that the storage buffer comprises thimerosal. Lim teaches storing its non-carboxylated latex particles with non-specific antibodies in the follow storage buffer: 0.1 M glycine, 0.9% sodium chloride buffer, 1% bovine serum albumin and 0.02% sodium azide. It does not teach using thimerosal in its storage buffer. Therefore, it does not teach each and every limitation recited in claim 26. Accordingly, reconsideration and withdrawal are respectfully requested.

Rejection under 35 U.S.C. § 103

Claims 23-27 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Nilsson *et al.* "Microparticles for selective protein determination in capillary electrophoresis," ELECTROPHORESIS, (2001) 22: 2384-2390 ("Nilsson") and Salzman *et al.* (WO 01/040280) ("Salzman"), in view of Sukosol *et al.*, "Fusion protein of Solmonella typhi flagellin as antigen for diagnosis of typhoid fever," ASIAN PACIFIC J. OF ALLERGY AND IMMUN., (1994) 12:21-25 ("Sukosol").

A. Recited Invention

The present invention is an agglutination test using latex particles coated with gamma-globulin fraction of serum prepared against S. typhi specific flagellin gene product. Thus, the results can be observed with the naked-eye, and without the aide of any instrument. Consequently, the recited invention can be used in the field.

To this end, claim 23 recites a process for the preparation of an agglutination reagent for rapid and early detection of typhoid comprising preparing antibody specific to a Flagellin gene of *Salmonella typhi*, preparing latex particles suspension, and coating of the latex particles with the antibody. The antibody is prepared by raising a hyper immune sera against a purified protein encoded by a Flagellin gene specific to *Salmonella typhi*.

The latex particle suspension is prepared in part by mixing 1% carboxylated latex particles and 40 mM 2-N morpholinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1, washing twice with 20 mM MES buffer of pH 5.5 thereby forming a washed latex particle. 1-ethyl-3 (3-dimethyl-amino propyl) carbodiimide hydrochloride (EDC) in 20 mM MES buffer of pH 5.5 is added to the washed latex particle.

The washed latex particle is reacted with the antibody fraction. The reaction is stopped by adding 1M glycvine (pH 11.0). Thereafter, the antibody coated latex particle is washed with 50 nM glyvine (pH 9.5), 0.03% surfactant and 0.05% sodium azide.

Claim 24 is directed to an agglutination reagent for rapid and early detection of typhoid. The reagent comprises a carboxylated latex particle coated with an antibody specific to a Flagellin gene of *Salmonella typhi*. The particles are stored in a storage buffer. Claims 25-27 depend from claim 24 and further define the particle, storage buffer and antibody.

B. Cited References

Nilsson is directed to a system for detecting protein using two different monoclonal antibodies for human chorionic gonadotropin ("hCG") (Nilsson at abstract). There are two different monoclonal antibodies covalently bound to the latex particles (Nilsson at abstract). Once the latex particles are created, Nilsson teaches washing the particles with Tris-BSA and blocking the carboxyl group with Tris-HCL (Nilsson at page 2385). When a test reagent is mixed with hCG containing sample, an immune complex is formed (Nilsson at abstract). The complex is separated from the latex particles using capillary electrophoresis and detected by UV-Vis detection system (Nilsson at abstract). Since Nilsson discusses using an instrument to detect the hCG, the system is confined to the laboratory, and cannot be used in the field. A capillary electrophoresis is different from an agglutination system.

Nilsson does not disclose several of the limitations recited in claim 23. Namely, Nilsson does not disclose: an antibody specific to *Salmonella typhi* Flagellin gene product because Nilsson is directed to hCG, (4) washing the polyclonal monospecific coated latex particle with MES because Nilsson teaches washing with Tris-BSA, and blocking the carboxyl

group with Tris-HCl, or (5) an agglutination reagent because Nilsson teaches capillary electrophoresis.

Nilsson also fails to disclose several limitations recited in claims 24-27. Namely, it fails to disclose: (1) the recited antibody, (2) an agglutination reagent, and (3) the storage buffer.

Salzman generally is directed to a polypeptide derived from flagellin polypeptides used to generated an immune response to gram-negative bacteria (Salzman at abstract). Salzman used a portion of *Salmonella muenchen* specific flagellin gene product to prepare an antibody (Salzman at pages 11-21). The gene product is comprised of less than 160 amino acids, which also match the flagellin amino acid sequence of other gram negative bacteria. Salzman used GST as a tag with the gene sequence to make the fusion protein (Salzman at page 31). Antibodies raised against this protein will not only react with clinical sample of *Salmonella muenchen* and other gram-negative bacteria, but will also react with parasitic infections caused by *Schistosoma japonicum*. Therefore, Salzman does not teach a polyclonal antibody, nor a monospecific antibody because the antibody generated according to Salzman's disclosure will not be specific to *Salmonella muechen*.

Salzman also does not disclose several of the limitations recited in the claim. Namely, Salzman does not disclose (1) the recited antibody raised against Salzman's protein will react with *Salmonella muenchen*, other gram-negative bacteria and parasites such as *Schistosoma japonicum*, (2) using a latex particle, or (3) the recited reagents.

Sukosol used a 900 base pair gene sequence specific to the *Salmonella typhi* flagellin gene to make a fusion protein with a GST tag in a vector for detection of the antibody (IgM) in serum samples of individuals suspected to have typhoid or related infection (Sukosol at page 23, column 3). Sukosol does not disclose using the *Salmonella typhi* flagellin gene product for making an antibody, binding the antibody to a latex particle or using the antibody in an agglutination reagent.

C. Argument

When making a rejection under 35 U.S.C. § 103, the Examiner has the burden of establishing a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, the prior art must be evaluated based on what it, as a whole, teaches to one of ordinary skill in the art. To establish this, each and every claimed element must be taught or made obvious by the applied references. Additionally, there must be some reason to combine the references in a manner that results in the recited invention.

Here, there is no reason to combine the references to result in a latex particle coated with the recited antibody. Furthermore, the references to do teach or suggest the recited blocking and washing steps, or the storage buffer.

Point I. There is no reason to eliminate Nilsson's second antibody

There is no reason why one would reasonably expect Nilsson's invention to work if the second antibody is removed from the latex particle. Assuming that one was motivated to combine the references, one would create a monoclonal antibody raised against Sukosol's 900 base pair gene sequence specific to *Salmonella typhi*. This antibody would be reacted with Nilsson's latex particle. This latex particle also includes a second antibody.

In contrast, claim 23 recites that the latex particle is coated with a polyclonal-monospecific antibody specific to *Salmonella typhi*, not two different antibodies. Likewise, claims 24-27 recite that the latex particle is coated with an antibody, not two different antibodies. Since there is no reason for one to have removed the second antibody from Nilsson's latex particle, the invention is not obvious over the cited references.

Point II. There is no reason to use a polyclonal antibody instead of a monoclonal one.

Furthermore, there is no reason to use a polyclonal antibody instead of a monoclonal antibody. Nilsson and Salzman teach using a monoclonal antibody on its latex particles. Sukosol provides no motiviation to use a polyclonal antibody instead. Since such a reason is not provided, the recited invention is patentable over the cited references.

Point III. There is no reason to substitute Nilsson's blocking and washing steps with the recited ones.

Additionally, the cited references do not teach the recited blocking and washing steps, and there is no reason to substitute Nilsson's washing and blocking steps with the recited washing step. Nilsson teaches blocking the residual activated carboxyl groups with 0.1 M Trus-HCl, pH 8.0 containing 0.2% BSA, and washing the antibody coated latex particles with Tris-BSA. However, claim 23 recites that the reaction between the latex particle coated and the polyclonal-monospecific antibody is stopped with 1M glycine (pH 11.0), and the polyclonal-monospecific antibody coated latex particle is washed with 50 mM glycine, pH 8.5; 0.03% surfactant and 0.05% sodium azide.

There is no reason for one to substitute Nilsson's blocking and washing steps for the ones recited in claim 23. Accordingly, the recited invention is patentable over the cited references.

Point IV. There is no teaching or reason to use the recited storage buffer.

Nilsson teaches storing its coated latex particles in Tris-BSA. The references do not teach the storage buffer recited in claim 24 and further defined in claim 26. Without such a teaching, there cannot be a reason to modify the cited references in a manner that leads one to the recited invention.

Point V. The secondary evidence rebuts any *prima facie* case of obviousness.

Notwithstanding the reasons set-forth above, even assuming that a *prima facie* case of obviousness has been established, the secondary evidence of long-felt but unresolved need, failure of others and commercial success rebuts the rejection. Typhoid is one of the most prevalent diseases inflicting countries in tropical regions of the world. It is also a very difficult disease to diagnose. The gold standard for diagnosing Typhoid is by taking a culture. However, this diagnostic test is very time consuming. Other methods include the Widal test, Widal slide agglutination Test, Radioimmunoassay, ELISA based antigen detection methods and commercially available Typhidot™. These methods all have several shortcomings, as listed in Table 1.

Table 1: Shortcomings of Typhoid Tests

Test	Shortcoming
Widal Test	Takes 18-25 hours after 6-7 days of enteric fever[6]
Widal Slide Agglutination Test	Take 1-3 min. after 6-7 days of enteric fever[7]
Culture Test	Takes 3-14 days after first day of fever[8]
ELISA based test	Takes 8 hours after 6-7 days of enteric fever
Radioimmunoassay	Very complicated and involve the use of radioactive material, which is not available in many countries where Typhiod is a health concern.[9]

[6] Specification at page 2, lines 5-6.
[7] Specification at page 2, lines 5-6.
[8] Specification at page 2, line 20 to page 3, line 16
[9] Specification at page 3, line 17 to page 4, line 3.

In contrast to these shortcomings, the claimed invention provides a process for the preparation of an agglutination reagent that can be used to detect typhoid early and rapidly with 100% specificity. The recited invention takes approximately 1-2 minutes to develop a color positive sample.[10] Since the invention relies on seeing a color, an untrained person can read the results. Thus, the invention is fast and easy to use in comparison to the prior art.

Furthermore, the invention can be used to detect typhoid at the onset of infection. The prior art is not capable of doing this.

Additionally, the invention is highly specific and sensitive for detecting typhoid. In contrast, the Widal test cross-reacts with other febrile organisms and other organisms within the Enterobacteriaceae family.[11] Also, it provides false positives when administered to a person who has been vaccinated, or when the appropriate baseline level of typhoid for a region is not known (typhoid, being an endemic, causes background level of antibody in endemic areas).[12] The culturing technique is also a low sensitivity test for typhoid (40 – 60 %) because there are very few organisms in circulation (as low as 1 / ml), which leads to false negatives.[13]

The agglutination reagent is stable for more than nine months at 4°C.

Finally, a leading Indian company has already taken interest in this invention.

Thus, there is a long-felt, but unresolved need for a fast and easy typhoid diagnostic test that can detect typhoid in its early stages. The prior art only teaches tests that are either time consuming (e.g. culture tests), or can only detect typhoid 6-7 days after enteric fever has been observed. Others who have tried to address this problem have failed by either developing a test that is time consuming, or can only detect typhoid 6-7 days after enteric fever.

[10] Specification at page 7, lines 17-19.
[11] Specification at page 1, lines 24-26.
[12] Specification at page 2, lines 1-4.
[13] Specification at page 3, lines 8-10.

In view of this secondary evidence, Applicants have rebutted any *prima facie* case of obviousness set-forth by the Examiner. In the absence of evidence to the contrary, the Applicants respectfully request that this rejection be reconsidered and withdrawn.

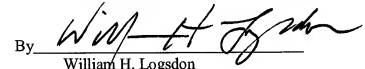
Conclusion

Due to the differences discussed above, a combination of the cited references do not result in the recited invention.

For these reasons, Applicants respectfully request reconsideration and withdrawal of the objections and rejections, allowance of pending claims 23-27, and rejoinder of claim 28.

Respectfully submitted,

THE WEBB LAW FIRM

By 

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